REMARKS

Upon entry of the above amendments, claims 28-42 are pending in the application.

Rejections Under 35 U.S.C. § 103

I. Claims 28, 30 and 32

Claims 28, 30 and 32 are rejected under 35 U.S.C. §103(a) for allegedly being unpatentable over U.S. Pat. No. 4,193,990 ("the '990 patent") and U.S. Pat. No. 4,193,991 ("the '991 patent") in view of Pratelli *et al., J. Vet. Diag. Invest. 11*: 365-7 (1999) ("Pratelli *et al.*"). Although *none* of the references teach or suggest the claimed MVC vaccine, the Examiner alleges the following:

> It would have been *prima facie* obvious to the person of ordinary skill in the art at the time the invention was made to make a vaccine for . . . [MVC]. The person of ordinary skill in the art would have been motivated to make a vaccine for . . . [MVC] because Pratelli teaches the importance of the canine pathogen, and reasonably would have expected success because . . . [the '990 and/or '991 patent(s)] made a vaccine from a virulent strain of CPV. Furthermore, the widespread presence of antibodies to CPV-1 in the canine population indicates that dogs are able to mount an effective immune response to CPV-1.

Office Action, page 4, lines 13-19. Applicants respectfully disagree with the Examiner's assessment.

MVC is a totally different virus than CPV-2. Attached as Exhibit A are pages from the third edition (2006) of Craig E. Greene's authoritative treatise "Infectious Diseases of the Dog and Cat." On page 70, under **Etiology**, it states that "CPV-1 [a.k.a. MVC] is distinctly differentiated from CPV-2 by its host cell range, spectra of hemagglutination, genomic properties, and antigenicity. . . . CPV-1 and CPV-2 are different viruses; no homology in DNA-restriction sites between the two viruses has been demonstrated using several restriction enzymes." Under **Diagnosis**, it states that "CPV-1 [a.k.a. MVC] will not cross react with any of the serologic or fecal detection methods for CPV-2." *Id.*

That CPV-1 represents an entirely different virus from CPV-2 is corroborated by the documents identified by the Examiner in a Notice of References Cited PTO-892 form

accompanying an Office Action mailed on June 29, 2006. Schwartz, D. *et al., Virology 302*: 219-223 (2002) ("Schwartz *et al.*") states that "MVC . . .[is] antigenically and genetically distinct form the canine parvovirus type-2 (CPV) based on antibody cross reactivity and restriction enzyme analysis of the viral DNA . . . ." See page 219, left column, first paragraph. Moreover, as evident from Schwartz *et al.*, the skilled artisan perceives CPV-1 to be distinct from CPV-2: "Here we show that MVC is a distinct member of the Parvoviridae which is most closely related to the bovine parvovirus, *although it shares only 43% identity in DNA sequence with that virus.*" See page 222, top of left column, emphasis added.

There is a clear genetic difference between MVC/CPV-1 and CPV-2. In fact, Schwartz *et al.* seems to suggest in the last paragraph in the left column of page 222 a better way of grouping the class of parvoviruses: i) adenoassociated viruses, ii) rodent virus-related viruses, and iii) erythroviruses. Whereas the second group includes canine parvovirus (i.e., CPV-2), the authors include MVC in the last group. Moreover, the authors caution that this grouping is imperfect because "MVC is still only distantly related to the other viruses, indicating that it diverged in the distant past." *See* sentence bridging left and right columns on page 222.

Other publications cited by the Examiner also corroborate the fact that MVC is a totally different virus than CPV-2. Pratelli *et al., J. Vet. Diag. Invest. 11*: 365-7 (1999) was cited by the Examiner in the PTO-892 form accompanying the Office Action mailed on March 21, 2007. This publication states that "[a]ntigenic and genomic properties of MVC are distinct from those of canine parvovirus type 2 (CPV-2). . . ." *See* page 365, left column. Truyen, U., Recent Advances in Canine Infectious Diseases, International Veterniary Information Service (January 2000) was also cited by the Examiner in the same PTO-892 form. This publication states that "[t]wo distinct parvoviruses (CPV), are now known to infect dogs- the pathogenic CPV-2, . . . and the 'minute virus of canines' (MVC, CPV-1). . . . MVC, *a completely different parvovirus*, had not been associated with natural disease until 1992." *See* first paragraph, emphasis added.

Both CPV-1/MVC and CPV-2 happen to have been initially classified as parvoviruses. This initial taxonomic classification appears to form the principal basis of the present rejection alleging the obviousness of Applicants' claimed vaccine. Various publications, however, clearly

indicate that skilled artisans recognize that MVC and CPV-2 are entirely different viruses: they are genetically and antigenically distinct and diverged from each other in the distant past. CPV-2 has been widely studied to the point where numerous vaccines are on the market. In contrast, prior to Applicants' invention, no MVC vaccines were available. Hence, it is unreasonable to expect that MVC can be made into a vaccine simply because vaccines exist for CPV-2.

Finally, Applicants question the relevancy of the Examiner's statement regarding "the widespread presence of antibodies to CPV-1 in the canine population indicates that dogs are able to mount an effective immune response to CPV-1." Office Action, page 4, lines 17-19. The fact that canines have been naturally afflicted by MVC and have antibodies to this virus does not anticipate or render obvious Applicants' vaccine. Greene (Exhibit A) clearly shows that MVC is a problem for dogs:

> Once a diagnosis has been made, treatment of pups suffering CPV-1 infection is unrewarding because of the rapid progression of the disease. However, mortality may be reduced by ensuring that the environmental temperature of newborn pups is kept warm and by adequate nutrition and hydration. No vaccine is available at present.

See page 70, right column under **Therapy and Prevention.** Hence. CPV-1 is a problem amongst pups despite any widespread presence of antibodies to CPV01 in the canine population.

Claims 28, 30 and 32 each require the presence of an MVC vaccine. Since none of the applied publications individually or combined teach or suggest an MVC vaccine, Applicants respectfully request that the Examiner reconsider and withdraw this rejection.

II. Claims 28- 33

Claims 28-33 are rejected under 35 U.S.C. §103(a) for allegedly being unpatentable over the '990 and '991 patents in view of Pratelli *et al.*, and further in view of U.S. Pat. No. 6,159,477 ("the '477 patent"). Although *none* of the references teach or suggest the claimed MVC vaccine, the Examiner alleges the following:

> It would have been *prima facie* obvious to the person of ordinary skill in the art at the time the invention was made to prepare a multivalent vaccine for canine pathogens. The person of ordinary skill in the art would have been motivated to add antigens from different pathogens because multivalent vaccine

> for dogs are well known in the art and [the '477 patent] teaches vaccinating dogs against different canine pathogens in one vaccine.

Office Action, page 5, lines 12-16. Applicants respectfully disagree with the Examiner's assessment.

Both CPV-1/MVC and CPV-2 happen to have been initially classified as parvoviruses. As indicated above and fully incorporated here, present research and skill in the art, however, clearly recognize that these two viral types are entirely different: they are genetically and antigenically distinct and diverged from each other in the distant past. CPV-2 has been widely studied to the point where numerous vaccines are on the market. In contrast, prior to Applicants' invention, no MVC vaccines were available. Hence, it is unreasonable to expect that MVC can be made into a vaccine simply because vaccines exist for CPV-2.

Claims 28-33 each require the presence of an MVC vaccine. Since none of the applied publications individually or combined teach or suggest an MVC vaccine, Applicants respectfully request that the Examiner reconsider and withdraw this rejection.

III. Claims 28-42

Claims 28-42 are rejected under 35 U.S.C. §103(a) for allegedly being unpatentable over the '990 and '991 patents in view of Pratelli *et al.*, and the '477 patent, and further in view of Poulet *et al., Vet. Record 148:*691-5(2001) ("Poulet *et al.*") and Correa, Alabama Cooperative Extension System, November 2002 ("Correa"). Although *none* of the references teach or suggest the claimed MVC vaccine, the Examiner alleges the following:

> It would have been *prima facie* obvious to the person of ordinary skill in the art at the time the invention was made to vaccinate the bitch and allow the puppies to nurse within 24 hours. The person of ordinary skill in the art would have been motivated to vaccinate the pregnant bitch because Poulet teaches the importance for survival of the puppies. The person of ordinary skill in the art would have also been motivated to allow the puppies to nurse in the first 24 hours because Correa teaches the importance of puppies receiving colostrums to absorb antibodies to protect from diseases.
>
> The combination of references teaches the limitations of the instant claimed invention.

Office Action, page 6, line 16 to page 7, line 1. Applicants respectfully disagree with the Examiner's assessment.

Both CPV-1/MVC and CPV-2 happen to have been initially classified as parvoviruses. As indicated above and fully incorporated here, present research and skill in the art, however, clearly recognize that these two viral types are entirely different: they are genetically and antigenically distinct and diverged from each other in the distant past. CPV-2 has been widely studied to the point where numerous vaccines are on the market. In contrast, prior to Applicants' invention, no MVC vaccines were available. Hence, it is unreasonable to expect that MVC can be made into a vaccine simply because vaccines exist for CPV-2.

Claims 28-42 each require the presence of an MVC vaccine. Since none of the applied publications individually or combined teach or suggest an MVC vaccine, Applicants respectfully request that the Examiner reconsider and withdraw this rejection.

IV. Other Points

The Examiner dismisses the shortcomings of the applied publications and the differences between MVC and CPV-2 on the following grounds:

> The prior art shows that dogs commonly possess antibodies to CPV-1. The fact that severe pathology appears to occur mostly in pups (with undeveloped immune systems) further suggested that adults are able to mount an effective immune response to the virus. Therefore it would have been prima facie obvious to immunize pregnant bitches in order to provide a high level of anti-CPV-1 antibodies in colostrum to protect vulnerable pups. It would have been obvious to use attenuated or inactivated virus because it was know that the virus itself can induce a protective immune response in adult dogs.

Office Action, page 7, lines 6-12.

The Examiner's reasoning ignores the fact that although MVC was discovered about 40 years ago and has long been recognized to cause severe pathology, no MVC vaccine has yet been developed. Prior to Applicants' invention, MVC vaccines were neither taught or suggested. This can be attributed to the fact that MVC is an entirely different virus from CPV-2, each having different genetic, antigenic and other properties. Applicants' claimed vaccines are novel and non-

obvious regardless of whether canines have been naturally afflicted by MVC and/or naturally have antibodies to this virus. Applicants' vaccines are not naturally occurring.

Conclusion

Applicants do not believe that any other fee is due in connection with this filing. If, however, Applicants do owe any such fee(s), the Commissioner is hereby authorized to charge the fee(s) to Deposit Account No. 02-2334. In addition, if there is ever any other fee deficiency or overpayment under 37 C.F.R. §1.16 or 1.17 in connection with this patent application, the Commissioner is hereby authorized to charge such deficiency or overpayment to Deposit Account No. 02-2334.

Applicants submit that this application is in condition for allowance, and request that it be allowed. The Examiner is requested to call the Undersigned if any issues arise that can be addressed over the phone to expedite examination of this application.

Respectfully submitted,

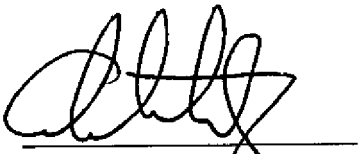

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EXHIBIT A

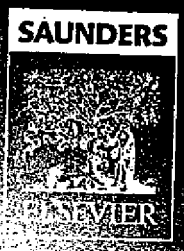
Infectious Diseases

OF THE DOG AND CAT

THIRD EDITION

Craig E. Greene, DVM, MS, DACVIM

Professor Emeritus and Josiah Meigs Distinguished Teaching Professor
Department of Infectious Diseases and Department of Small Animal Medicine
College of Veterinary Medicine
The University of Georgia
Athens, Georgia



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INFECTIOUS DISEASES OF THE DOG AND CAT, edition 3

ISBN-13: 978-1-4160-3600-5
ISBN-10: 1-4160-3600-8

Notice

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ISBN-13: 978-1-4160-3600-5
ISBN-10: 1-4160-3600-8

Publishing Director: Linda Duncan
Acquisitions Editor: Anthony Winkel
Developmental Editor: Shelly Stringer
Publishing Services Manager: Julie Eddy
Project Manager: JoAnn Amore
Designer: Julia Dummitt

Printed in Canada

Last digit is the print number: 9 8 7 6 5 4 3 2

SUGGESTED READINGS*

6. Evermann J. 1985. Paramyxovirus infections of dogs. *Vet Rec* 117:450-451.
9. Macartney L, Cornwell HJ, McCandlish IA, et al. 1985. Isolation of a novel paramyxovirus from a dog with enteric disease, *Vet Rec* 117:205-207.
12. Vieler E, Herbst W, Baumgartner W, et al. 1994. Isolation of a parainfluenza virus type 2 from the prostatic fluid of a dog, *Vet Rec* 135:384-385.

*See the CD-ROM for a complete list of references.

CHAPTER • 8

Canine Viral Enteritis

Dudley L. McCaw and Johnny D. Hoskins

Since the late 1970s, viral enteritis has become recognized as one of the most common causes of infectious diarrhea in dogs younger than 6 months. Canine parvovirus (CPV)–1 and –2), canine coronavirus (CCV), and canine rotaviruses (CRVs) have been incriminated as primary pathogens. Astrovirus, herpesvirus, enteroviruses, calicivirus, parainfluenza viruses, and viruslike particles have been isolated from or identified in feces from dogs with diarrhea, but their pathogenicity is uncertain.[21,29,51]

CANINE PARVOVIRAL ENTERITIS

Etiology

CPVs are small, nonenveloped, DNA–containing viruses that require rapidly dividing cells for replication (Fig. 8-1). As is the case with all parvoviruses, CPV-2 and -1 are extremely stable and are resistant to adverse environmental influences. CPV-2 is known to persist on inanimate objects, such as clothing, food pans, and cage floors, for 5 months or longer.

Most common detergents and disinfectants fail to inactivate CPVs. A noteworthy exception is sodium hypochlorite (1 part common household bleach to 30 parts water), which is an effective and inexpensive disinfectant. It is important that exposure to this disinfectant be prolonged (at least 10 minutes) and thorough.

Canine parvoviral enteritis is probably one of the most common infectious disorders of dogs. This highly contagious, often fatal disease is caused by CPV-2. Since its emergence in the late 1970s, CPV-2 has undergone genetic alterations in the dog, with development of new strains of the virus.[90,91,93] In 1980, the original strain of CPV-2 evolved into type 2a (CPV-2a); and in 1984, another variant designated type 2b (CPV-2b) appeared. These CPV-2 alterations were associated with a genetic adaptation, enabling the parvovirus to replicate and spread more effectively in susceptible dogs. In the United States and Japan, CPV-2b has largely replaced those previously isolated strains, whereas in the Far East[10,68] and Europe,[17,26] both CPV-2a and -2b predominate.[24] In 2000, another strain was reported (CPV-2c), which was an adaptation that allowed infection of cats.[44] Although CPV-2c has been isolated only from leopard cats, infection in domestic cats and dogs is likely.[44] Genetic mutations in the structure of the surface transferrin receptor (TfR) of the virus has resulted in structural alterations that control the host adaptation of CPV strains.[41] For a further discussion of CPV strains in cats, see Canine Parvovirus Infection of Cats in Chapter 10.

Epidemiology

Natural CPV-2 infections have been reported in domestic dogs, bush dogs *(Speothos venaticus)*, coyotes *(Canis latrans)*, crab-eating foxes *(Cerdocyon thous)*, and maned wolves *(Chrysocyon brachyurus)*; and most if not all *Canidae* are susceptible. Experimental infections can be produced in domestic ferrets, mink, and cats; however, the infection is generally self-limiting. The original CPV-2 isolates produced only systemic and intestinal infections in dogs,[128] whereas the newer type 2a and 2b strains may infect felines under experimental[68,73,131] and natural[71,127] circumstances (see Chapter 10). In domestic dogs, CPV-2 infection does not necessarily result in apparent disease; many dogs that become naturally infected never develop overt clinical signs. When the disease occurs, clinical illness is most severe in young, rapidly growing pups that harbor intestinal helminths, protozoa, and certain enteric bacteria such as *Clostridium perfringens*, *Campylobacter* spp., and *Salmonella* spp. In susceptible animals, the incidence of severe disease and death can be very high.

CPV-2 is highly contagious, and most infections occur as a result of contact with contaminated feces in the environment. In addition, people, instruments (equipment in veterinary facilities or grooming operations), insects, and rodents can serve as vectors. Dogs may carry the virus on their hair coat for extended periods. The incubation period of CPV-2 in the field is 7 to 14 days; experimentally, the incubation period has been found to be 4 to 5 days. With CPV-2a and -2b strains, the incubation period in the field can be as brief as 4 to 6 days.

Acute CPV-2 enteritis can be seen in dogs of any breed, age, or sex. Nevertheless, pups between 6 weeks and 6 months of age, and Rottweilers, Doberman pinschers, Labrador retrievers, American Staffordshire terriers, German shepherds, and Alaskan sled dogs seem to have an increased risk.[25,39]

Pathogenesis

CPV-2 spreads rapidly from dog to dog via oronasal exposure to contaminated feces (Fig. 8-2). Virus replication begins in lymphoid tissue of the oropharynx, mesenteric lymph nodes, and thymus and is disseminated to the intestinal crypts of the

small intestine by means of viremia. Marked plasma viremia is observed 1 to 5 days after infection. Subsequent to the viremia, CPV-2 localizes predominantly in the gastrointestinal (GI) epithelium lining the tongue, oral and esophageal mucosae, and small intestine and lymphoid tissue, such as thymus, lymph nodes, and bone marrow. It may also be isolated from the lungs, spleen, liver, kidney, and myocardium.[138]

Normally, intestinal crypt epithelial cells mature in the small intestine and then migrate from the germinal epithelium of the intestinal crypts to the tips of the villi (Fig. 8-3, *A*). After reaching the villous tips, the intestinal epithelial cells acquire their absorptive capability and aid in assimilating nutrients. Parvovirus infects the germinal epithelium of the intestinal crypts, causing destruction and collapse of the epithelium (see Fig. 8-3, *B*). As a result, normal cell turnover (usually between 1 and 3 days in the small intestine) is impaired, and the villi become shortened. CPV-2 also destroys mitotically active precursors of circulating leukocytes and lymphoid cells. In severe infections, the results are often neutropenia and lymphopenia. Secondary bacterial infections from gram-negative and anaerobic microflora cause additional complications related to intestinal damage, bacteremia and endotoxemia, and disseminated intravascular coagulation (DIC).[84,129,130] Active excretion of CPV-2 begins on the third or fourth day after exposure, generally before overt clinical signs appear. CPV-2 is shed extensively in the feces for a maximum of 7 to 10 days. Development of local intestinal antibody is most likely important in the termination of fecal excretion of parvovirus. Serum antibody titers can be detected as early as 3 to 4 days after infection and may remain fairly constant for at least 1 year.

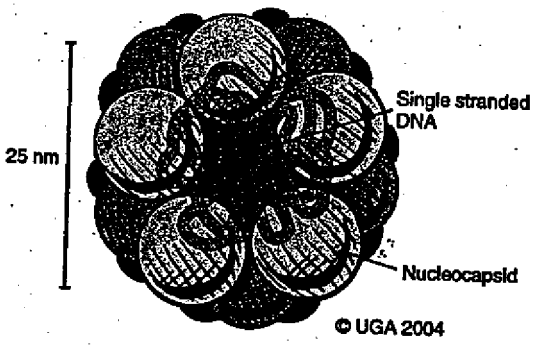

Fig 8-1 Structure of parvovirus. (Courtesy University of Georgia, Athens, Ga.)

Clinical Findings

CPV-2 infection has been associated with two main tissues—GI tract and myocardium—but the skin and nervous tissue can also be affected. In addition, other clinical complications of secondary infection or thrombosis can occur. A marked vari-

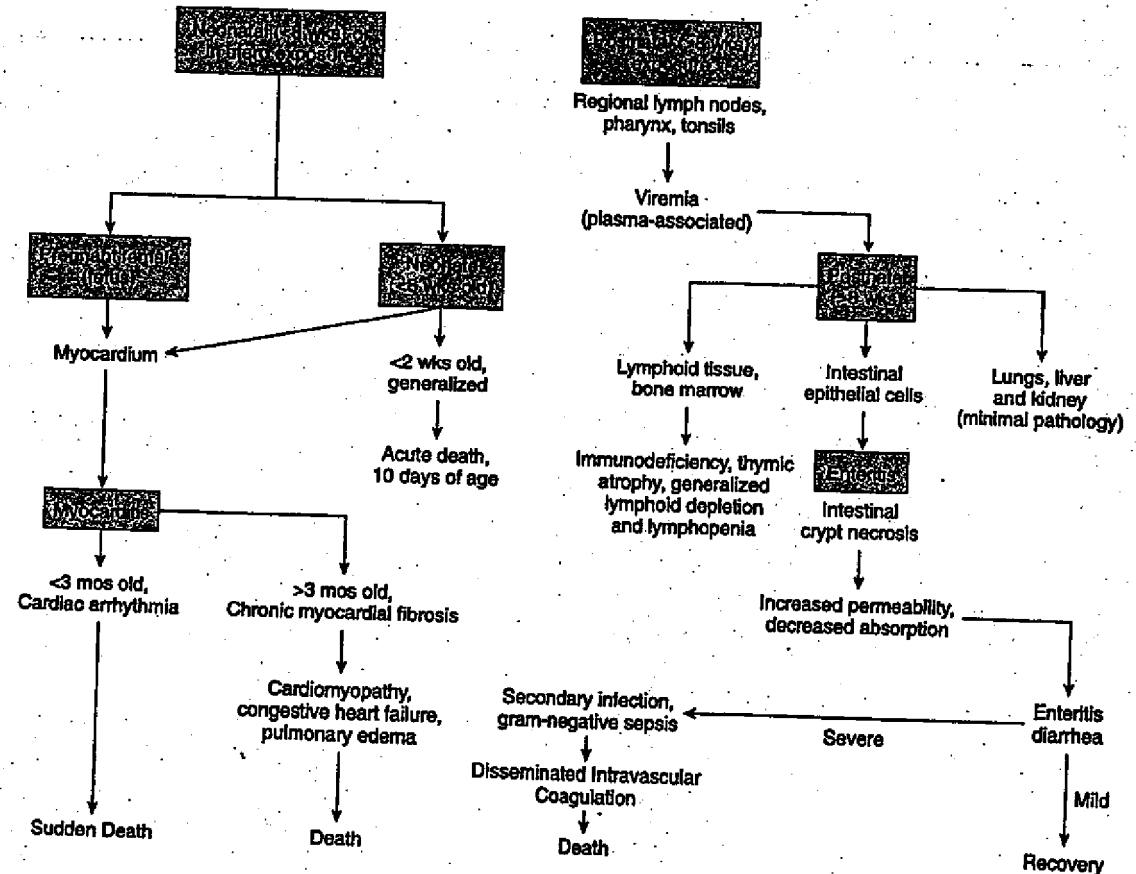

Fig 8-2 Sequential pathogenesis of CPV-2 infection.

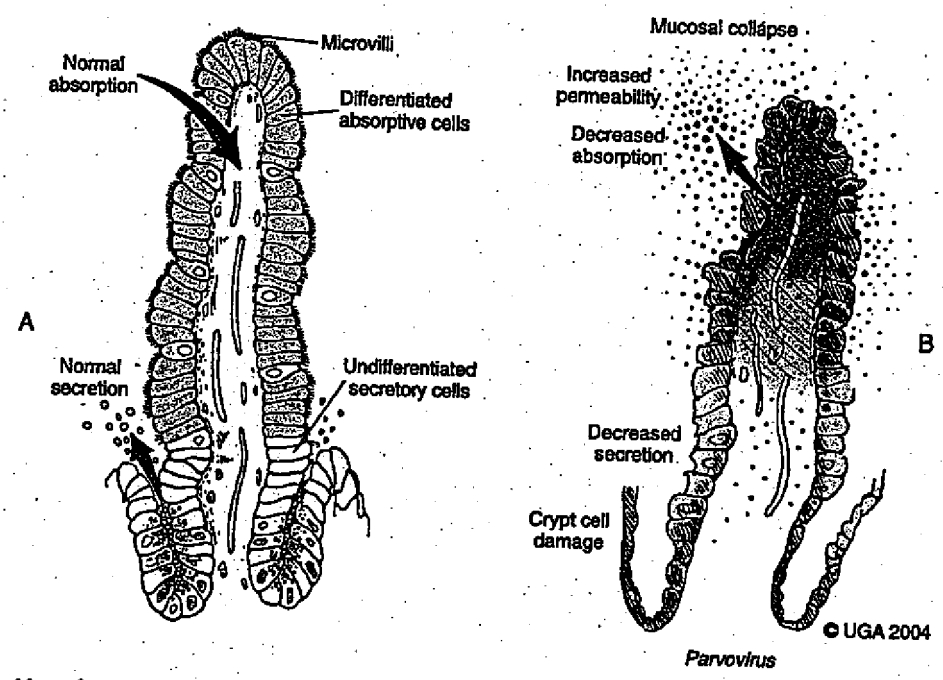

Fig 8-3 A, Normal intestinal villus showing cellular differentiation along the villus. B, Parvovirus-infected villus showing collapse and necrosis of intestinal villus. (Courtesy University of Georgia, Athens, Ga.)

ation is found in the clinical response of dogs to intestinal infection with CPV-2, ranging from inapparent infection to acute fatal disease. Inapparent, or subclinical, infection occurs in most dogs. Severity of the CPV-2 enteritis depends on the animal's age, stress level, breed, and immune status. The most severe infections are usually in pups younger than 12 weeks because these pups lack protective immunity and have an increased number of growing, dividing cells.

Parvoviral Enteritis

CPV-2 enteritis may progress rapidly, especially with the newer strains of CPV-2. Vomiting is often severe and is followed by diarrhea, anorexia, and rapid onset of dehydration. The feces appear yellow-gray and are streaked or darkened by blood (Fig. 8-4). Elevated rectal temperature (40° to 41° C [104° to 105° F]) and leukopenia may be present, especially in severe cases. Death can occur as early as 2 days after the onset of illness and is often associated with gram-negative sepsis or DIC, or both. Younger age, neutropenia, and Rottweiler breed have been associated with a poorer chance of survival (unpublished data).[31]

Neurologic Disease

Primary neurologic disease may be caused by CPV-2 but more commonly occurs as a result of hemorrhage into the central nervous system (CNS) from DIC or from hypoglycemia during the disease process, sepsis, or acid-base-electrolyte disturbances.[1] Concurrent infection with viruses such as canine distemper virus is also possible. Cerebellar hypoplasia, common in kittens prenatally or neonatally infected with feline panleukopenia virus, has not been adequately reported in pups with CPV-2 infection. CPV DNA was amplified using polymerase chain reaction (PCR) from brain tissue of two dogs with cerebellar hypoplasia, but time of exposure to CPV was not mentioned.[114] CPV-2 has been identified in the CNS of cats (see Central Nervous System Infection in Chapter 10).

Fig 8-4 Dog with severe bloody diarrhea characteristic of severe parvoviral enteritis. (Courtesy University of Georgia, Athens, Ga.)

Cutaneous Disease

Erythema multiforme was diagnosed in a dog with parvoviral enteritis.[20] Skin lesions included ulceration of the footpads, pressure points, and mouth and vaginal mucosa. Vesicles in the oral cavity and erythematous patches on the abdomen and perivulvar skin were also present. Parvovirus was confirmed in the affected cells by immunohistochemistry.

Canine parvovirus–2 Myocarditis

CPV-2 myocarditis can develop from infection in utero or in pups younger than 8 weeks. All pups in a litter are usually affected. Pups with CPV-2 myocarditis often die, or they succumb after a short episode of dyspnea, crying, and retching. Signs of cardiac dysfunction may be preceded by the enteric form of the disease or may occur suddenly, without apparent previous illness. The spectrum of myocardial disease in individuals is wide and may include any of the following: acute diarrhea and death, without cardiac signs; diarrhea and apparent recovery followed by death, which occurs weeks or months later as a result of congestive heart failure; or sudden onset of congestive heart failure, which occurs in apparently normal pups at 6 weeks to 6 months of age. Myocarditis is still occasionally found in pups born to isolated, unvaccinated bitches[135] in contrast to its frequent occurrence during the widespread epizootic outbreaks of the late 1970s in CPV-naive dogs.[1] Myocarditis, with or without enteritis, has been associated with natural CPV-2a and -2b infections in 6- to 14-week-old dogs from Korea.[139] CPV infection appears not to be a common cause of heart disease because PCR analysis at necropsy of 27 dogs with either dilated cardiomyopathy or myocarditis did not detect CPV in any of the samples.[63]

Thrombosis

Dogs with naturally occurring CPV-2 infections have clinical and laboratory evidence of hypercoagulability.[86] These dogs may develop thrombosis or phlebitis with catheters or visceral thrombi.

Bacteriuria

Asymptomatic urinary tract infection has been detected in approximately 25% of pups following CPV-2 enteritis.[50] This predisposition was attributed to fecal contamination of the external genitalia in association with neutropenia. Untreated subclinical urinary tract infection may lead to chronic urinary infection as an undesirable consequence.

Intravenous Catheter Infection

Bacteria from GI or environmental origin have been isolated from the intravenous (IV) catheters removed from dogs being treated for suspected parvoviral infections.[55] Most of these organisms were gram-negative types (*Serratia, Acinobacter, Citrobacter, Klebsiella,* and *Escherichia*). Most organisms were resistant to penicillins, first-generation cephalosporins, and macrolides while being susceptible to aminoglycosides, fluoroquinolones, chloramphenicol, potentiated sulfonamides, and clavulanate-potentiated penicillins. Despite the positive culture results of the catheter tips, none of the dogs showed systemic clinical signs of infection, and only one developed local phlebitis.

Diagnosis

The sudden onset of foul-smelling, bloody diarrhea in a young (under 2 years) dog is often considered indicative of CPV-2 infection. However, all dogs with bloody diarrhea (with or without vomiting) are not infected necessarily with CPV-2. Other enteropathogenic bacterial infections should also be considered (see Chapter 39). All clinical signs characteristic of CPV-2 infection are seldom present at any one time. Leukopenia, although not found in all dogs, is usually proportional to the severity of illness and the stage of disease at the time the blood is taken. Abnormal coagulation test results may include prolongation of the activated partial thromboplastin time, increased thromboelastogram amplitude, and decreased antithrombin III activity.[86]

Fecal enzyme-linked immunosorbent assay (ELISA) antigen tests are available for in-hospital testing for CPV-2 infection (see Appendix 6). These tests are relatively sensitive and specific for detecting CPV-2 infection.[38,44,45,52] However, the period of fecal virus shedding is brief; CPV-2 is seldom detectable by 10 to 12 days after natural infection. This corresponds to 5 to 7 days of clinical illness. Positive results confirm infection or may be induced by all attenuated live CPV-2 vaccines (vaccine virus can yield a false-positive result in dogs 5 to 12 days after vaccination); negative results do not eliminate the possibility of CPV-2 infection. Generally, vaccine-induced reactions are weak positive compared with natural infection.

CPV-2 typically produces lesions in the jejunum, ileum, mesenteric lymph nodes, and other lymphoid tissues. CPV-2 can be isolated from these tissues or feces using tissue culture systems, if performed early. Later in the course of disease, virions become coated by antibodies and cleared. In most tissues, intranuclear inclusions are observed. In the glossal epithelium, these may appear as being within the cytoplasm, when in fact they originate in the nuclear space.[42] Immunochemical methods can also be used to detect virus in tissue culture, electron microscopy (EM) scan of feces or tissues (see Pathologic Findings in this chapter). PCR has been used as a specific and sensitive means of detecting CPV in feces of infected dogs.[68,73,132] This method can also help to differentiate between virulent and vaccine CPV strains.[118]

As a general rule, parvoviruses cause hemagglutination of erythrocytes. Inhibition of hemagglutination by CPV-2 antisera can be used to demonstrate serum antibody. The presence of high hemagglutination inhibition (HI) titer in a single serum sample collected after the dog has been clinically ill for 3 or more days is diagnostic for CPV-2 infection. Rising titers (seroconversion) can also be demonstrated when acute and 10- to 14-day convalescent serum samples are compared using either canine or feline parvovirus in HI and virus neutralization (VN) tests. ELISA tests are also available that permit distinction between IgG and IgM.[111] In-office ELISA test kits are commercially available for semiquantitative IgG and IgM measurements (Immunocomb, Biogal Labs, Megiddo, Israel)[137,136] and for determining adequate IgG titers for vaccination (CPV/CDV Test Kit, Synbiotics, San Diego, Calif). See Appendix 6 for further information on these products.

Pathologic Findings

Early lesions are most pronounced in the distal duodenum; later, the jejunum is more severely affected. The intestinal wall is generally thickened and segmentally discolored, with denudation of intestinal mucosa and the presence of dark, sometimes bloody, watery material within the stomach and intestinal lumen (Fig. 8-5). In mild cases, the lesions are not easy to distinguish from those of nonspecific enteritis. Enlargement and edema of thoracic or abdominal lymph nodes have been observed.

The intestinal lesions are characterized by necrosis of the crypt epithelium in the small intestine. Intranuclear viral inclusion bodies may be seen in these epithelial cells and throughout the squamous epithelia of the upper GI tract.[62] The pathologic changes may range from mild inflammation to diffuse hemorrhagic enteritis. The villi are shortened or obliterated, owing to lack of epithelial replacement by maturing crypt cells, resulting in collapse of the lamina propria (Fig. 8-6). Necrosis and depletion of the lymphoid tissue (e.g., Peyer's patches; mesenteric lymph nodes, thymus, and spleen) are present. Pulmonary edema or alveolitis may be observed in dogs dying of complicating septicemia.[130] Histologic examination is usually definitive; however, specific identification of parvovirus in tissue specimens can be done by immunofluorescence or other immunochemical methods. Using indirect fluorescent antibody (FA) testing, antigen in dogs with

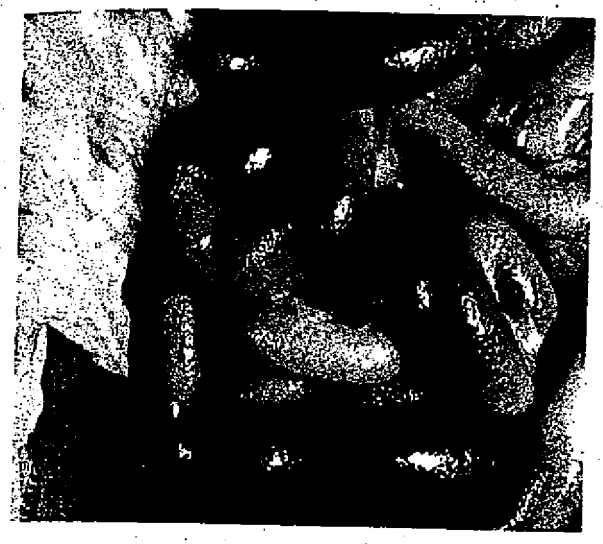

Fig 8-5 Small intestine at necropsy from a dog that died suddenly of parvoviral enteritis. Note the discoloration of the intestinal wall and fibrin on the serosal surfaces. (Courtesy Veterinary Pathology, University of Georgia, Athens, Ga.)

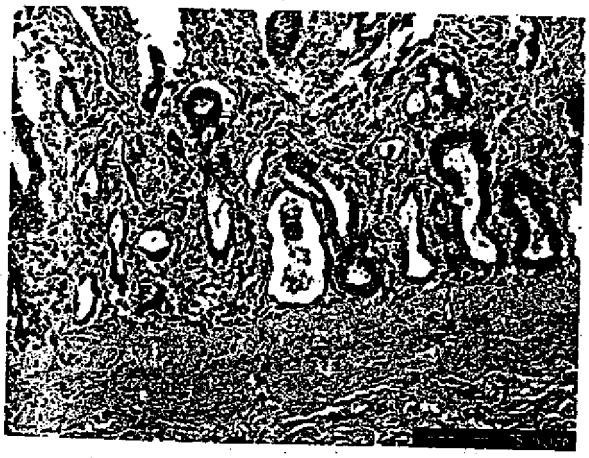

Fig 8-6 Photomicrograph of the small intestine of a dog that died of parvoviral enteritis. Villi are collapsed, and crypt lumina are dilated and filled with necrotic debris (H and E stain, ×100). (Courtesy Barry Harmon, University of Georgia, Athens, Ga.)

lethal CPV enteritis can be found in the dorsal side of the tongue (96.3%), pharynx (81%), esophagus (50%), ventral tongue (20.4%), planum nasale (5.6%), small intestinal mucosa (85.2%), bone marrow (81.6%), spleen (79.6%), thymus (66.7%), mesenteric nodes (50.4%), palatine tonsils (58.5%), and myocardium (1.9%).[138] In situ hybridization is a valuable specific tool for virus identification in formalin-fixed or wax-embedded tissue specimen.[134]

Parvoviral myocarditis, when present, is recognized grossly as pale streaks in the myocardium (Fig. 8-7). The myocardial lesions consist of a nonsuppurative myocarditis with multifocal infiltration of lymphocytes and plasma cells within the myocardium. Basophilic intranuclear inclusion bodies have been observed in cardiac muscle fibers, and parvolike

Fig 8-7 Heart from a dog that died of the myocardial form of CPV-2 infection. Pale streaking of the myocardium is apparent. A similar lesion will be noted with CPV-1 infection in puppies younger than 3 weeks. (Courtesy Pfizer Animal Health, Lincoln, Neb.)

virus particles have been demonstrated by EM and by in situ hybridization[135] in the inclusion bodies.

Therapy

The primary goals of symptomatic treatment for CPV-2 enteritis are restoration of fluid and electrolyte balance and preventing secondary bacterial infections. Antimicrobial agents, motility modifiers, and antiemetic agents are given in Table 8-1. Fluid therapy is probably the single most important aspect of clinical management and should be continued for as long as vomiting or diarrhea (or both) persists. Hypoglycemia and hypokalemia are common and should be corrected through additions to the IV fluids. Antimicrobial agents are recommended because the combination of severe disruption of the intestinal epithelium allowing bacteria into the blood and peripheral neutropenia increases the risk of sepsis.[108] The most common bacteria appear to be *Escherichia coli* and *Clostridium perfringens*.[129,130] The best antibacterial spectrum is provided by combination of a penicillin and an aminoglycoside. Before a nephrotoxic drug such as an aminoglycoside is administered, the patient should be fully hydrated. Antiemetic drugs are helpful to reduce fluid loss and decrease patient distress and allows for enteral nutrition. Metoclopramide hydrochloride and prochlorperazine have proved helpful in most dogs with persistent vomiting. The serotonin receptor antagonists are the most efficacious antiemetics.[57,83] Ondansetron and dolasetron have both been used in dogs. Drug therapy to alter gut motility is seldom recommended in the treatment of CPV-2 enteritis. If needed, narcotic antispasmodics (e.g., diphenoxylate hydrochloride, loperamide hydrochloride) are preferred when motility modifiers are needed.

Although withholding food and water are general recommendations in treating GI diseases, including parvovirus enteritis, recent information suggests this is not necessary. When dogs with parvovirus enteritis were fed beginning on the first day of treatment (via nasoesophageal tube), their recovery time was shortened, and they maintained body weight when compared with dogs that were treated the conventional method of withholding food until signs had ceased for 12 hours.[75]

After GI signs abate, a broad-spectrum dewormer and treatment for *Giardia* infection should be given. During the

Table 8-1

Drug Therapy for Canine Viral Enteritis

DRUG	DOSAGE[a] (mg/kg)	ROUTE	INTERVAL (HR)	DURATION (DAYS)
Antiemetic Agents				
Chlorpromazine	0.5	IM	8	prn
	1.0	Rectally	8	prn
	0.05	IV	8	prn
Metoclopramide	0.2–0.4	SC	8	prn
	1–2	IV[b]	24	prn
Prochlorperazine	0.1	IM	6–8	prn
Ondansetron	0.1–0.15	IV	6–12	prn
Dolasetron	1	IV, PO	24	prn
Antimicrobial Agents				
Ampicillin	10–20	IV, IM, SC	6–8	3–5
Cefazolin	22	IV, IM	8	3–5
Ceftiofur	2.2–4.4	SC	12	3–5
Gentamicin	2	IM, SC	8	3–5
Interferon-ω	2.5×10^6 units/kg	IV	24	3
Gastric Protectants				
Cimetidine	5–10	IM, IV	6–8	prn
Ranitidine	2–4	SC, IV	6–8	prn
Miscellaneous Therapy				
Whole blood	10–20 ml/kg	IV[c]		prn
Plasma	10–20 ml/kg	IV[c]		prn
Dexamethasone sodium phosphate[c]	2–4	IV		Do not repeat
Flunixin meglamine[c]	1	IV		Do not repeat
Antiendotoxin serum[d]	8.8 ml/kg (diluted in equal amount crystalloid fluid)	IV		Do not repeat
Colloid fluid[e]	20 ml/kg	IV	24	prn

IM, Intramuscular; *IV*, intravenous; *SC*, subcutaneous; *PO*, by mouth, *prn*, as needed.
[a]Dose per administration at specified interval. For additional information on these drugs, see Drug Formulary, Appendix 8.
[b]Slow infusion can be used for severe vomiting.
[c]Administered after correction of dehydration.
[d]Administered over 4 hours.
[e]SEPTI-SERUM; Immvac Inc., Columbia, MO. (Based on a concentration of >320 mg of IgG/ml.)
[f]Hetastarch or Dextran 70.

initial stage of CPV-2 enteritis, recommended adjunctive therapy has included transfusion of specific hyperimmune plasma or administration of antiendotoxin sera[18] (see Passive Immunization, Chapter 100, and Drug Formulary, Appendix 8). These adjuncts reportedly decrease mortality and the length of hospitalization[18] but are expensive. A recombinant bactericidal-permeability–increasing (BPI) protein, which counteracts endotoxin, did not alter clinical outcome or survival in dogs naturally infected with CPV-2.[85] This result is despite increases in plasma endotoxin in affected animals.

Recombinant human granulocyte colony-stimulating factor (G-CSF) has been advocated for the treatment of severe neutropenias induced by CPV-2 infection.[22] However, supplementing recombinant human G-CSF to neutropenic pups with CPV-2 infection did not change any aspect of their clinical outcome.[67,109,110] The lack of efficacy of exogenous G-CSF is probably the result of already existent high levels of endogenous G-CSF that are maximally stimulating the production of neutrophils.[11]

Dogs with experimental and natural parvovirus infection have been treated with recombinant feline IFN-ω in high IV dosages (2.5×10^6 units/kg) beginning early (4 days or less after infection) in the course of parvoviral infection.[15, 45,60,66] Reduced signs of clinical illness and mortality were observed in treated dogs. See Drug Formulary, Appendix 8 for further information on its availability and usage.

Several therapies have been recommended and empirically would seem of benefit, but they have not been examined well enough to indicate that they are efficacious.[56] Some puppies are severely anemic, which may be the result of GI loss of blood caused by the parvovirus enteritis, or it might be unrelated to parvovirus such as parasitism. Transfusion of whole blood might benefit these puppies. Hypoproteinemia is present in some puppies. A whole blood transfusion will help resolve the problem, but if erythrocytes are not needed, a more appropriate therapy is plasma transfusion. Ideally, serum albumin concentration should be maintained at 2.0 g/dl or greater. If edema is present as a result of decreased proteins and is not corrected by a plasma transfusion, then synthetic colloid such as hetastarch should be considered. Colloids should not be given until dehydration is corrected. Glucocorticoids and flunixin meglumine may have beneficial effects in

treating early sepsis or endotoxemia. These agents should not be used until dehydration is corrected, and repeated doses should not be given.

The use of hyperimmune plasma might be questioned because, at the time of clinical signs, the levels of antibodies are generally increased. However, pups that had a delayed or lower response are often more severely affected. Canine lyophilized IgG has been beneficial in treatment of dogs with naturally occurring CPV-2 infection.[58] Compared with control dogs, those receiving IgG as adjunctive therapy had reduced severity of disease, reduced cost of treatment, and reduced hospitalization time.

Pups that survive the first 3 to 4 days of CPV-2 enteritis usually make a rapid recovery, generally within 1 week in uncomplicated cases. Severely ill pups that develop secondary sepsis or other complications may require prolonged hospitalization.

Prevention

Immunity After Infection

A puppy that recovers from CPV-2 enteritis is immune to reinfection for at least 20 months and possibly for life. On reexposure to the various strains of CPV-2, protected pups will not have increased serologic titers, show overt signs of illness, or shed virus in the feces. In general, a good correlation exists between serum antibody titer, determined by either HI or VN testing, and resistance to infection. Serum antibody titers remain high for a prolonged period after CPV-2 enteritis, even if reexposure does not occur. If serum antibody titers become low, a localized infection is possible, but viremia and generalized illness are unlikely to develop. Although it may help in protection against entry of CPV-2, intestinal secretory IgA probably does not play a role in the longevity of protective immunity because intestinally derived antibody titers do not persist for longer than 15 days after infection.

Immunization and Duration of Immunity

Inactivated CPV-2 vaccines of sufficient antigenic mass protect dogs against wild-type CPV-2 exposure. If protective immunity is defined as complete resistance to subclinical infection, then that produced by most inactivated CPV-2 vaccines is short lived. Dogs vaccinated with inactivated CPV-2 vaccine can become subclinically infected as early as 2 weeks after vaccination. If a dog is given sequential doses of inactivated CPV-2 vaccine, however, a rapid secondary immune response is mounted, and the dog is protected for as long as 15 months.

Commercially prepared attenuated live and inactivated CPV-2 vaccines are available. These vaccines produce varying levels of protective immunity and are safe either alone or in combination with other vaccine components. Transient lymphopenia occurs 4 to 6 days after the administration of some attenuated live CPV-2 vaccines. Most attenuated live CPV vaccine strains replicate in the intestinal tract and are briefly shed in the feces. Although concern has been expressed about the possibility of attenuated CPV-2 vaccine undergoing reversion of virulence and causing apparent disease, experimental studies have shown that modified live virus (MLV) CPV-2 vaccines are safe.[49] The events following administration of attenuated live CPV-2 vaccines parallel those following wild-type CPV-2 infection. On day 2 after subcutaneous (SC) administration of vaccine, viremia and systemic distribution occur with shedding from GI tract on days 3 to 10. One difference between vaccine-induced and wild-type infections is that lower quantities of virus are shed after vaccination. Humoral immune responses to attenuated live vaccines that have been studied are similar to those observed with wild-type infection.

Serum antibody is usually detectable 3 days after vaccination, with levels rising rapidly to those observed after subsequent natural infection. Even if reexposure does not occur, protective antibody titers may persist for at least 2 years, and dogs exposed during this time should not become infected. Vaccination with potentiated attenuated CPV-2 vaccine has been shown to protect dogs on subsequent experimental challenge exposure.[115] On the basis of serum antibody titers, in a veterinary hospital setting, 27% of the dogs being evaluated for revaccination had titers below the protective level for CPV-2.[65] Although serum antibody titers are not absolute indicators of protection, they have a good correlation with protection against CPV-2 infection (see also Canine Parvoviral Infection, Chapter 100). Even systemic chemotherapy for neoplasia in dogs did not affect serum CPV-2 antibody titers.[33]

Attenuated Live CPV-2 Immunization

Contrary to publicized information, vaccination failure is not related to strain differences between field and vaccine strains. The primary causes of failure of vaccines are interfering levels of maternal antibody to CPV-2[82,96] and lack of sufficient seroconversion to the CPV-2 vaccine administered. The age at which pups can be successfully immunized is proportional to the antibody titer of the bitch, effectiveness of colostral transfer of maternal antibody within the first 24 hours of life, and immunogenicity and antigen titer of the CPV-2 vaccine. Pups from a bitch with low protective titer of antibody to CPV-2 can be successfully immunized by 6 weeks of age, but in pups from a bitch with a very high titer to CPV-2, maternal antibody may persist longer.[96]

Without knowledge of the antibody status of each puppy, recommending a practical vaccination schedule that will protect all of them is difficult. In addition, pups become susceptible to wild-type CPV-2 infection 2 to 3 weeks before they can be immunized. No vaccines are available that completely eliminate this window of susceptibility before pups become immunized.[96] With the *potentiated* vaccines presently available, which are more immunogenic than the original or *conventional* CPV vaccines, low levels of maternal antibody will not prevent successful response. Pups of unknown immune status can be vaccinated with a high-titer–attenuated live CPV-2 vaccine at 6, 9, and 12 weeks of age and then revaccinated annually.[37] A check for serum antibody level or an additional vaccination might be done at 15 to 16 weeks of age, especially in breeds that are at increased risk for CPV-2 enteritis.[13] See discussion of parvoviral infection in Chapter 100 for additional information.

Attenuated Live Canine Parvovirus-2b Immunization

Although not currently commercially available, experimental use of a modified live vaccine derived from CPV-2b produced higher antibody titers to CPV-2b and CPV-2 than did a vaccine derived from CPV-2.[102] In addition, the CPV-2b vaccine was able to produce a titer increase in puppies with higher maternal antibody levels.[101]

Experimental Vaccines

A large number of genetically engineered vaccines have been developed in an attempt to improve the protection afforded by inactivated products while reducing the antigenicity of the potentiated vaccines. A DNA vaccine containing a plasmid encoding the full length of the viral protein (VP)1 region of CPV-2 protected 9-month-old pups from clinical signs and fecal shedding of virus experimental challenge-infection.[48] A vaccine based on a recombinant plant virus expressing the VP2 peptide, coded by a subset of the VP1 gene, protected against clinical disease, with limited fecal shedding following challenge.[53] Neither of these vaccines produced sterile immu-

nity as follows attenuated CPV-2 vaccination. Intranasal or SC vaccination of mice with a plant virus expressing a CPV-2 peptide elicited systemic and mucosal antibody responses.[79,80]

Husbandry
CPV is one of the most resistant viruses to infect dogs. As a result, the hair coat and environment of the ill dog become contaminated. Diluted household bleach (1:30) with water should be applied to tolerable surfaces or used as a dip for animals leaving isolation facilities. Bleach should be added to washing of all utensils and bedding. The solutions require a 10-minute minimum exposure time. The shedding period is so short (under 4 to 5 days following the onset of illness) that the environment is of major concern. The virus can persist for months to years away from sunlight and disinfectants. Steam cleaning can be used for instantaneous disinfection of surfaces that do not tolerate hypochlorite. For further information on disinfection, see Chapter 94.

Public Health Considerations
Studies have failed to find any evidence of human infection by CPV-2, even among kennel workers in heavily contaminated premises, although people apparently can act as passive transport vehicles for the virus between dogs. Although CPV-2 is not itself a human pathogen, extra care should always be practiced in handling fecal materials from diarrheic animals.

CANINE PARVOVIRUS-1 INFECTION

Etiology
In 1967, CPV-1 (also referred to as minute virus of canines [MVC]) was first isolated from the feces of military dogs. Physical and chemical properties of CPV-1 are typical of parvoviruses. CPV-1 is distinctly differentiated from CPV-2 by its host cell range, spectra of hemagglutination, genomic properties, and antigenicity.[7] Using genetic analysis, it is most closely related to bovine parvovirus.[117]

CPV-1 can be propagated on the Walter Reed canine (WRC) cell line. By HI tests, CPV-1 is serologically distinct from parvoviruses of a number of other species. Apparently, CPV-1 and CPV-2 are different viruses; no homology in DNA-restriction sites between the two viruses has been demonstrated using several restriction enzymes.

Epidemiology
The domestic dog is the only proven host, although other Canidae are likely susceptible. Before 1985, CPV-1 was considered a nonpathogenic parvovirus of dogs. Since that time, clinical infections of CPV-1 in neonatal pups have been encountered by practicing veterinarians and diagnostic laboratory personnel. Serologic evidence indicates that its distribution is widespread in the dog population but is usually restricted to causing clinical disease in pups younger than 3 weeks,[8] but disease has been reported in pups 5 weeks of age.[100] A reasonable assumption is that the spread is similar to that of CPV-2. Although it was first identified in the United States, isolations have been made worldwide,[69] and similar to CPV-2, it is likely ubiquitous.

Pathogenesis
The virulence of CPV-1 for dogs is uncertain; however, it has been identified by immunoelectron microscopy in the feces of pups and dogs with mild diarrhea. Between 4 and 6 days after oral exposure, CPV-1 can be recovered from the small intestine, spleen, mesenteric lymph nodes, and thymus. Histologic changes in lymphoid tissue are similar to those observed in pups infected with CPV-2 but less severe. In addition, CPV-1 is capable of crossing the placenta and producing early fetal death and birth defects.[7] Experimental oronasal infection of neonatal specific pathogen-free (SPF) pups, with laboratory isolates from pups dying of enteric illness, produced only mild respiratory disease.[8] Naturally induced disease in young pups has been characterized by enteritis, pneumonia, and myocarditis.[47] Naturally infected dogs have been shown to have a reduction in both numbers and killing activity of phagocytes.[14]

Clinical Findings
CPV-1 has been observed infrequently in field dogs with mild diarrhea, as well as in the feces of clinically healthy animals. Primarily, CPV-1 infection is a cause of enteritis, pneumonitis, myocarditis, and lymphadenitis in pups between 5 and 21 days of age.[31] Many of these pups have mild or vague symptoms and eventually die, being classified as "fading pups." Affected pups usually have diarrhea, vomiting, and dyspnea and are constantly crying. Some puppies have respiratory disease with no enteric signs.[100] Sudden death with few premonitory signs has also been observed. Because of transplacental infections, this virus can cause failure to conceive or fetal death or abortion.

Diagnosis
CPV-1 infection should be considered in young (under 8-week-old) pups with mild diarrhea that clinically or histologically resemble CPV-2 disease but are serologically CPV-2 negative, or in unexplained fetal abnormalities, in abortions, or in fading pups. CPV-1 will not cross react with any of the serologic or fecal detection methods for CPV-2. EM has observed CPV-1 in fecal and rectal swab samples from field dogs. Immunoelectron microscopy is necessary to distinguish CPV-1 from CPV-2. Inhibition of hemagglutinating activity in stool suspensions by specific antiserum is also diagnostic for CPV-1. To determine exposure, sera can be tested for specific antibody with VN or HI tests. Because only the WRC cell line supports growth of CPV-1, the availability of virus isolation and serum VN tests is limited.

Pathologic Findings
Pathologic changes in nursing pups have included thymic edema and atrophy, enlarged lymph nodes, pasty soft stool in the intestinal tract, and pale gray streaks and irregular areas deep within the myocardium as found with CPV-2 (see Fig. 8-7). Histopathologic lesions are predominantly restricted to large intranuclear epithelial inclusions at the tips of the villi in the duodenum and jejunum. These inclusions are eosinophilic and often fill the nuclei. Other intestinal changes noted include crypt epithelial hyperplasia and single-cell necrosis of crypt epithelial cells. Lesions seen in other tissue include moderate to marked depletion or necrosis (or both) of lymphoid cells of Peyer's patches and thymus, severe pneumonitis with exudate in airways, and mineralized focal to diffuse areas of myocardial necrosis with cellular infiltration.

Therapy and Prevention
Once a diagnosis has been made, treatment of pups suffering CPV-1 infection is unrewarding because of the rapid progression of the disease. However, mortality may be reduced by ensuring that the environmental temperature of newborn pups is kept warm and by adequate nutrition and hydration. No vaccine is available at present.

Public Health Considerations
No known public health concern exists; however, extra care should always be practiced in handling sick pups and fecal

material from diarrheic animals because other enteropathogens may be present.

CANINE CORONAVIRAL ENTERITIS

Etiology

CCV is a member of the virus family *Coronaviridae* belonging to the order *Nidovirales* (Fig. 8-8). Different coronaviruses of this family infect a large number of species, including humans, cattle, swine, dogs, cats, horses, poultry, rats, and mice (see Table 11-1). To date, several strains of CCV have been isolated from outbreaks of diarrheal disease in dogs. The virus genome is composed of a single-stranded RNA chain; replication occurs in the cell cytoplasm of the host. Coronaviruses are fairly resistant and can remain infectious for longer periods outdoors at frozen temperatures. The virus loses infectivity in feces after approximately 40 hours at room temperature (20° C) and 60 hours when refrigerated (4° C).[123] Coronaviruses can be inactivated by most commercial detergents and disinfectants.

Epidemiology

In 1971, a CCV was isolated from feces of military dogs that were suffering from suspected infectious enteritis. Since then, several outbreaks of contagious enteritis have occurred and a similar coronavirus has been isolated. The true importance of CCV as a cause of infectious enteritis in dogs is unknown; however, CCV was genetically detected[3] or isolated[70] from 16% or 57%, respectively, of dogs with diarrhea in Japan. Serologic testing of Australian dogs showing signs of diarrhea revealed that 85% were positive for CCV–IgM antibodies, which indicates recent infection.[78] Serologic information suggests that CCV has been present indefinitely in the dog population and is an infrequent cause of infectious enteritis. CCV is highly contagious and spreads rapidly through groups of susceptible dogs. Neonatal pups are more severely affected than those of weaning age and adult dogs. CCV is shed in the feces of infected dogs for weeks to months or longer, and fecal contamination of the environment is the primary source for its transmission via ingestion.[124]

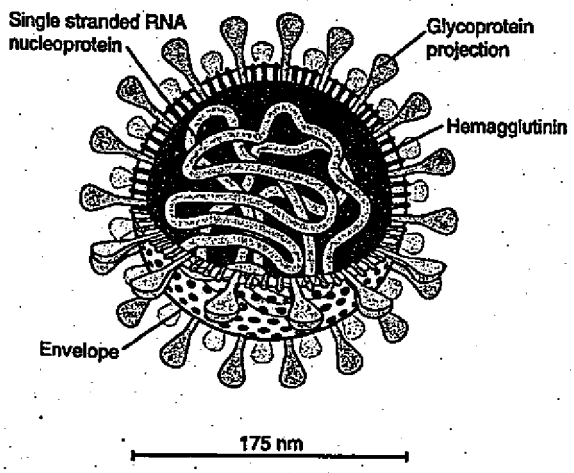

Fig 8-8 Structure of coronavirus. (Courtesy University of Georgia, Athens, Ga.)

Pathogenesis

The incubation period is short: 1 to 4 days in the field and only 24 to 48 hours experimentally. CCV can generally be isolated from the feces of infected dogs between 3 and approximately 14 days after infection.

After ingestion, CCV goes to the mature epithelial cells of the villi of the small intestine.[124,125] After uptake of CCV by M cells in the dome epithelium of Peyer's patches virus and viral antigen is transported to the underlying lymphoid tissue. Uptake in the gut lymphoid tissue suggests that CCV may persist or become latent in dogs, similar to the situation for feline coronavirus. Genetic analysis suggested that nucleotide substitutions occurred in the transmembrane protein M gene during the time of clinical illness.[104] The virus also rapidly reproduces within epithelial cells and accumulates within cytoplasmic vacuoles. Virions from these vacuoles may be released directly into the external environment via the apical plasmalemma or may be released after lysis of the apical cytoplasm of infected cells. After production of mature virus, infected cells develop severe cytoplasmic changes, and the microvilli of the brush border become short, distorted, and lost. The overall result is that infected cells become lost from the villi at an accelerated rate and are replaced by increased replication rate of immature cells in the crypts of the mucosa. Crypt epithelium is not destroyed; on the contrary, hyperplasia develops. Affected villi become covered by low columnar to cuboidal epithelium, show variable levels of villous atrophy and fusion, and become infiltrated by mononuclear cells in the lamina propria. Unlike CPV infection, villius necrosis and hemorrhage are rare.

Dogs can have CCV and CPV infections simultaneously, and some studies suggest that CCV infection enhances the severity of CPV infection. Conversely, three of four puppies in a litter died from CCV enteritis 2 weeks after surviving CPV enteritis.[107] Concurrent infections with canine adenovirus-1 and CCV was suspected as the cause of severe enteric disease in an animal shelter.[104] Other enteropathogens such as *Clostridium perfringens, Campylobacter* spp., *Helicobacter* spp., and *Salmonella* spp. may increase the severity of CCV illness (see Chapter 39).

Clinical Findings

Differentiating CCV from other infectious causes of enteritis is difficult. Theories suggest that CCV infection is usually less dramatic than CPV-2 infection. The clinical signs can vary greatly, and dogs of any breed, age, and sex are affected. This finding contrasts with CPV infections in which affected dogs are usually younger than 2 years. Infected dogs usually have a sudden onset of diarrhea preceded sometimes by vomiting. Feces are characteristically orange in color, very malodorous, and infrequently contain blood. Loss of appetite and lethargy are also common signs. Unlike CPV-2 infection, fever is not constant, and leukopenia is not a recognized feature.

In severe cases, diarrhea can become watery, and dehydration and electrolyte imbalances can follow. Concurrent ocular and nasal discharges have been noted, but their relationship to the primary infection is unknown. Most of the dogs affected recover spontaneously after 8 to 10 days. When secondary complicating factors are present (parasites, bacteria, or other viruses), the disease can be significantly prolonged.

Diagnosis

Making a definitive diagnosis of CCV-induced disease is difficult. EM can detect CCV in fresh feces. Approximately 1×10^6 virions are needed in unconcentrated fecal samples for identification of CCV by EM; thus false-negative findings are possible. Viral isolation is difficult because CCV does not grow well in tissue or cell culture systems. A highly sensitive reverse transcriptase PCR has been developed to detect CCV in fecal

specimens.[28,99,106] Serum VN and ELISA tests for CCV antibody have been developed.[111] Positive CCV serum titers of affected dogs can only confirm exposure to CCV, and serum IgG titers have no relationship to protection as do intestinal secretory IgA titers.

Pathologic Findings

Mild infections are grossly unremarkable. In severe cases, the intestinal loops are dilated and filled with thin, watery, green-yellow fecal material. Mesenteric lymph nodes are commonly enlarged and edematous.

Atrophy and fusion of intestinal villi and a deepening of the crypts characterize the intestinal lesions of CCV. Also present are an increase in cellularity of the lamina propria, flattening of surface epithelial cells, and discharge of goblet cells. With well-preserved tissues, FA staining can enable specific detection of virus in the intestinal lesions.

Therapy

Deaths associated with diarrheal disease are uncommon but occur in pups as a result of electrolyte and water loss with subsequent dehydration, acidosis, and shock. Management must emphasize supportive treatment to maintain fluid and electrolyte balance as described for CPV-2 infection. Although rarely indicated, broad-spectrum antimicrobial agents can be given to treat secondary bacterial infections. Good nursing care, including keeping the dogs quiet and warm, is certainly essential.

Prevention

Inactivated and MLV vaccines are available for protection against CCV infection.[23,87] Two doses 3 to 4 weeks apart and annual revaccination are recommended for immunization of dogs regardless of age. These vaccines are relatively safe but provide incomplete protection in that they reduce but do not completely eliminate replication of CCV in the intestinal tract after challenge.[88,89] Assessing the role of the CCV vaccines in protection against disease is difficult because CCV infections are usually inapparent or cause only mild signs of disease. For additional information on vaccination, see Coronaviral Infection in Chapter 100.

Public Health Considerations

CCV is not believed to infect people. Coronaviruses are not strictly host specific; thus the possibility of human infection cannot be excluded. For this reason, extra care should always be practiced in handling sick pups and fecal material from diarrheic animals.

CANINE ROTAVIRAL INFECTION

Etiology

Rotaviruses are recognized as important enteric pathogens in many animal species and in people. They are sometimes referred to as duovirus, reovirus-like, and rotalike virus agents. Currently, rotaviruses are classified as distinct members of the family *Reoviridae*. CRV is a double-stranded RNA, nonenveloped virus that is approximately 60 to 75 nm in diameter (Fig. 8-9). CRV is resistant to most environmental conditions outside the host.

Rotaviruses have been isolated in tissue cultures or observed by EM of specimens from many species, including mice, monkeys, calves, pigs, foals, lambs, humans, rabbits, deer, cats, and dogs.

Epidemiology

Rotaviruses are transmitted by fecal-oral contamination. The viruses are well adapted for survival outside the host and for passage through the upper GI tract. Serum antibodies to rotavirus have been identified in dogs and cats of all ages.

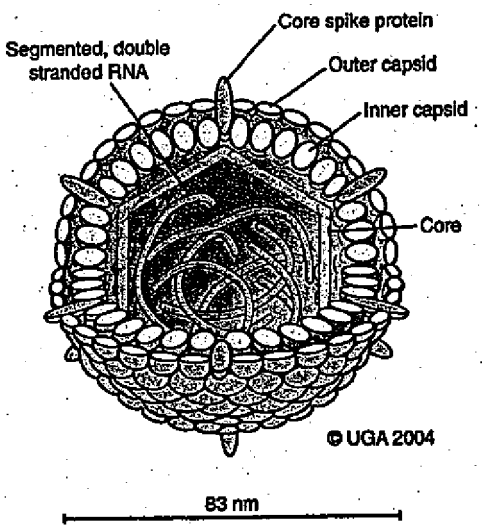

Fig 8-9 Structure of rotavirus. (Courtesy University of Georgia, Athens, Ga.)

Pathogenesis

Rotaviruses infect the most mature epithelial cells on the luminal tips of the small intestinal villi, leading to mild-to-moderate villous atrophy. Infected cells swell, degenerate, and desquamate into the intestinal lumen, where they release a large number of virions that become sources of infection for lower intestinal segments and for other animals. Necrosis of rotavirus-infected cells is most pronounced 18 to 48 hours after oral infection. Necrotic cells are rapidly replaced by immature crypt epithelium. Clinical signs result primarily from the villous atrophy, leading to mild to moderate maldigestion and malabsorption and osmotic diarrhea.

Clinical Findings

Most clinical rotaviral infections have been demonstrated in the feces of pups younger than 12 weeks, with mild diarrhea. Some cases of severe fatal enteritis associated with CRV have been reported to occur in pups as young as 2 weeks. The clinical signs are usually not as severe as those for the other canine enteric viruses (CPV-2 and CCV). A watery to mucoid diarrhea is usual, and this lasts for 8 to 10 days. The pups usually remain afebrile. CRV may contribute to enteric disease in mixed viral infections.

Diagnosis

Most pathogenic rotaviruses share common group-specific internal capsid antigens that can be detected by many methods, including commercial fecal ELISA (Rotazyme, Abbott Labs, N. Chicago, Ill.; Enzygnost, Behring Inst., Marburg, Germany) and latex agglutination (Rotalex, Orion Diag, Helsinki, Finland; Slidex Rota-kit, Biomerieux, Marcy-l'Etoile, France) tests used to diagnose human rotavirus infection (see also Appendix 6).[111] Rotaviruses can also be identified in fecal specimens by EM, although care must be taken to differentiate rotaviruses from the apparently nonpathogenic reoviruses occasionally present in dog feces. EM improves specificity of the test. Testing for seroconversion is possible but not widely available.

Pathologic Findings

Pathologic changes are limited to the small intestine, consisting of mild to moderate villous blunting. The virus can be detected in frozen sections by fluorescent antibody techniques.

Therapy and Prevention

Most dogs recover naturally from their infection. Treatment, if needed, consists solely of symptomatic therapy as described for CPV-2 enteritis. No vaccines are available for CRV, and current estimates of the frequency and severity of the disease do not appear to justify vaccine development.

Public Health Considerations

Rotaviruses are generally host specific; however, the various strains cannot be easily distinguished, and the possibility of human infection cannot be eliminated. Rotaviral infections in people usually occur in young infants and children (younger than 4 years). Poor sanitation and hygiene, as exist in developing countries, increase the prevalence of infection. Persons handling feces from diarrheic dogs should take routine precautions.

OTHER VIRAL ENTERITIDES

A large number of other viruses have been identified in feces of dogs both with and without diarrhea. For the most part, the pathogenicity and importance of these viruses as causes of infectious enteritis remain unknown. Based on work in other species, some viruses may be true enteric pathogens, whereas others are most likely incidental findings.

Astrovirus-like particles have been reported in the stools of clinically healthy and diarrheic dogs. Astroviruses are known to cause enteritis in other species, such as swine, but whether this is either true or common in the dog is unknown. The viruses have also been identified in diarrheic cats (see Chapter 12).

A herpesvirus antigenically related to feline herpesvirus has been isolated from a dog with diarrhea, but Koch's postulates have not been fulfilled.[51] Similarly, the importance of serologic reactivity of some dogs to human echoviruses and coxsackieviruses is unclear (see also Enteroviral Infections, Chapter 24).

An apparently specific canine calicivirus has been isolated on several occasions from the feces of dogs with enteritis, sometimes alone and sometimes in conjunction with other known enteric pathogens.[72,113] Similarly, an antigenically distinct parainfluenza virus, isolated from a dog with bloody diarrhea, was believed to be causal (see Chapter 7).

The study of viral enteritis in dogs is in its infancy. Undoubtedly, there are other viruses that affect the GI tract of dogs, but they remain to be discovered and characterized.

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*See the CD-ROM for a complete list of references.

CHAPTER • 9

Canine Viral Papillomatosis

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ETIOLOGY

The papillomavirus was first described in 1933 when Shope discovered the agent responsible for cutaneous papillomas in the cottontail rabbit.[19] Multiple canine papillomas in dogs are uncommon, comprising less than 12.5% of canine skin tumors.[20] Benign mucocutaneous tumors of epithelial origin are caused by infectious papillomavirus of the *Papovaviridae* family. The papilloma viruses are categorized with the polyomaviruses to form the papovaviruses. Members of this family are small (33 to 60 nm), naked, ether-resistant, double-stranded circular DNA tumor viruses, similar in structure to but larger than parvoviruses; they form crystalline structures within the nuclei of infected cells.[22,34] These viruses lack a lipid envelope and are acid stable and relatively thermostable, which may explain much of their inherent resistance.[34] Papillomaviruses are naturally oncogenic, producing benign warts, and are usually species and site specific. Cross-species infection of horses by bovine papillomaviruses type 1 and type 2 have been reported.[13] Most isolated viruses lack serologic cross-reactivity. Although antigenically distinct, papillomaviruses of humans, cattle, horses, dogs, and cats share at